LIV. THE CARBOHYDRATE AND FAT METABOLISM OF YEAST.

IV. THE NATURE OF THE PHOSPHOLIPINS.

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After yeast has been incubated in a well-oxygenated solution of glucose, fructose or sucrose, its fat-content becomes very considerably increased. If the solution in which the yeast is incubated contains not only sugar but also a mixture of alkali phosphates, the total amount of lipoid (ether-soluble matter) which can be extracted from the yeast after its incubation is double that which can be extracted from the same amount of yeast incubated in a similar sugar solution from which phosphates are absent [Smedley-MacLean and Hoffert, 1923]. After incubation in the sugar-phosphate medium the phosphate-content of the yeast is also greatly increased, the increase depending on the concentration of the *sugar* in the medium. After yeast has been incubated in a solution containing only alkali phosphates, no increase in the phosphate-content of the yeast is observed [Smedley-MacLean and Hoffert, 1924].

This effect of the addition of phosphates is very striking and the inference seems permissible that the phosphates themselves play some part in the transformation of carbohydrate to fat. Evidence was adduced to show that the first stage of this change probably consists in the formation of a hexose-phosphate.

Again, in the complex lipin molecule phosphoric acid is known to occur in combination with fatty acids. The relation of the fatty acid to the lipin molecule is still unknown: the fatty acids may first be formed and then built up into the lipin molecule, or perhaps the whole change from carbohydrate to fatty acid may take place while the various groups are associated together in some complex molecule of which phosphoric acid forms an integral part.

Since the formation of fat from carbohydrate in yeast can be very largely influenced by altering the constituents of the medium in which the yeast is incubated, it seemed important to examine the nature of the yeast phospholipins, the proportion which these bear to the other constituents of the lipoid matter and the changes which take place when the composition of the medium is altered, especially by the addition of phosphates.

Previous work on yeast lipins.

As early as 1866, Hoppe-Seyler [1866] showed that an ether-soluble substance was present in yeast containing both nitrogen and phosphorus. Nägeli denied this but Hoppe-Seyler [1879] published a further paper in which he showed that of the substance extracted by ether from yeast the unsaponifiable matter constituted 24·14 % and at least another 14 % consisted of lipin. From this lipin he isolated both choline and glycerophosphoric acid.

Koch [1902] showed that the ratio of methyl groups to nitrogen atoms, instead of being 3 : 1 as it is in lecithin, in the yeast lipin was only 1·3 : 1, a ratio very much more nearly akin to that found in kephalin. Koch argued therefore that choline could not be the chief base of yeast lipin.

Sedlmayer [1903] found choline and isolated palmitic acid but failed to find oleic acid. A preliminary note published by Austin [1924] indicated the presence of both lecithin and kephalin in yeast, since both choline and hydroxyethylamine were detected, thus confirming the previous finding of Koch.

The fatty acids of yeast fat have been examined by a number of observers and oleic, linolic and palmitic acids have been identified with certainty and evidence has been adduced that lauric acid is probably present; small amounts of a higher melting acid, possibly arachidic, have also been found [Hinsberg and Roos, 1904; Neville, 1913; Smedley-MacLean and Thomas, 1920].

Method of experiment.

A series of experiments was first carried out in order to determine the changes which occur in the phosphorus-content of pressed yeast and in the proportion of its unsaponifiable matter after the yeast has been incubated in the sugar solution and in the sugar-phosphate medium respectively.

A known weight of yeast was incubated for 24 hours in a litre of 4 % sugar solution and a similar quantity of yeast in a litre of the same solution to which 3·962 g. NaH_2PO_4 and 0·286 g. K_2HPO_4 had been added. A rapid current of oxygen was passed through both liquids; it is essential that the rate of oxygen current should be as nearly as possible the same in both experiments. The yeast was then filtered off and analysed. The easiest method of extracting the fat is to boil the yeast for two hours with normal hydrochloric acid and to extract the fat from the residue. The lipin is however decomposed by this method and it is therefore necessary to separate the fat from the yeast by repeatedly extracting the dried yeast with alcohol. It was generally found necessary to make from ten to twelve extractions of the yeast, using in each case from fifteen to twenty times its own weight of alcohol, the final extraction being carried out with boiling alcohol. The yeast was first treated with about twenty times its weight of 96 % alcohol to remove most of the water, the diluted alcohol filtered off, concentrated under diminished pressure to a small volume and the residue extracted with light petroleum. The alcohol from subsequent extractions was evaporated under diminished pressure and the residues taken up in light petroleum.

In some of these experiments we determined the percentage of phosphorus in the fat obtained from each successive extract; no distinct regularity was observed: on the whole the phosphorus-content of the fat was higher in the earlier fractions and there was certainly no indication that the phosphorised fat was more firmly bound or more difficult to extract from the cell than the fat or other lipoid constituents. Since lecithin and kephalin each contain about 4 % of phosphorus, the amount of phospholipin was calculated on this basis from the phosphorus-content of the fat.

The results are set out in the following table.

Table I.

No. of expt	Total lipoid by alcohol extraction g.	Total P in yeast g.	Total P in lipoid g.	% P in lipoid g.	% N calc. as protein	% unsap. matter
I. *Analysis of 15 grams of original pressed yeast.*						
833	0·1736	0·083	0·0023	1·32	1·42	—
835	0·1554	0·096	0·0020	1·29	1·83	—
837	0·1378	0·097	0·0003	0·22	1·58	—
839	0·1729	0·088	0·0018	1·06	1·72	—
841	0·1552	0·085	0·0019	1·23	1·69	—
845	0·1637	0·074	0·0019	1·14	1·69	31·1
847	0·1702	0·104	0·0021	1·21	1·53	35·4
Mean values	0·1613	0·090	0·0018	1·07	1·64	33·2
II. *Analysis of similar amount of yeast after incubation in 4 % glucose solution.*						
833	0·3320	0·098	0·0043	1·45	1·23	31·9
835	0·5031	0·106	0·0026	0·51	1·58	—
837	0·3508	0·098	0·0028	0·82	1·58	—
839	0·5002	0·090	0·0044	0·87	1·58	—
841	0·4080	0·091	0·0043	1·04	1·53	—
843	0·3740	0·079	0·0034	0·94	1·61	32·3
845	0·5455	0·093	0·0053	0·97	1·52	27·3
Mean values	0·4305	0·094	0·0039	0·94	1·52	30·5
III. *Analysis of similar amount of yeast after incubation in 4 % glucose-phosphate solution.*						
833	0·8235	0·134	0·0067	0·81	1·26	—
835	0·9192	0·146	0·0076	0·80	1·49	—
837	0·7630	0·154	0·0059	0·78	1·42	—
839	0·9172	0·144	0·0060	0·65	1·56	—
841	0·8254	0·143	0·0070	0·85	1·61	—
843	0·5770	0·120	0·0043	0·74	1·61	36·2
845	0·7794	0·127	0·0041	0·53	1·48	28·4
Mean values	0·8008	0·138	0·0058	0·74	1·49	32·3

From the figures given in the above table it follows that 100 g. of the original pressed yeast contain 1·08 g. of ether-soluble substance and 0·29 g. phospholipin, the latter being calculated from the phosphorus-content. The proportion of lipin in most of the samples examined was appreciably higher than this but one sample differed from the others in having an extremely low phosphorus-content and brought down the average amount.

After incubation in glucose solution alone, 100 g. of yeast contained 2·87 g. of total lipoid matter and 0·65 g. phospholipin. After incubation in the glucose-phosphate medium the ether-soluble substance constituted 5·34 % of the original weight of pressed yeast and contained 0·97 g. of phospholipin. The total amount of ether-soluble substance had increased to five times its original amount and that of phospholipin to three times its original value. The variations in the percentage of lipin found in the incubated yeasts were considerably less than those in the original samples of pressed yeast taken for investigation.

The variations in the amount of unsaponifiable matter were not sufficient and the number of determinations made were too few for us to be able to attach any significance to them. In all cases approximately 30 % of the ether-soluble matter consisted of unsaponifiable substance. Since the total amount of the unsaponifiable matter increases in proportion to the amount of fat formed and is often five or six times that originally present, it must have been made by the yeast from carbohydrate, and the process is aided by the presence of phosphate in the medium.

Preparation of ether-soluble material for the isolation of lipins.

As shown above, the average content of lipin in 100 g. of pressed yeast is only 0·30 g.: this is however raised to approximately 1 g. if the yeast be first incubated in the well oxygenated glucose-phosphate medium. We determined therefore to increase the proportion of lipoid substances in the yeast before extracting it with alcohol, by incubating it in a suitable medium. The long and tedious process of extracting a reasonable amount of starting material was thus expedited and a considerable saving of alcohol effected.

In one experiment 3 kg. of pressed yeast, obtained from liquid brewery yeast, well washed with water, were added to 30 gallons of a 4 % solution of cane sugar containing 0·369 g. Na_2HPO_4 and 0·0286 g. KH_2PO_4 %, and for 28 hours a rapid current of air was blown through the solution. The yeast was allowed to settle overnight and in the morning the solution was decanted, the yeast filtered through a cloth, rubbed up with alcohol and again filtered by suction. The pressed-out yeast was ground with one-quarter of its weight of sand and extracted five or six times with alcohol. In this way from 3 kg. of yeast about 100 g. of extract were obtained containing roughly 20 g. of lipin. Determination of the amount of fat in a sample of the same yeast after it had been hydrolysed, showed that it contained nearly 180 g. of lipoid matter.

Separation of phospholipins.

The phospholipin fraction was separated by repeatedly precipitating with acetone the ether solution of the total lipoid material, and was obtained as a white amorphous substance. Subsequently a further separation into lecithin and kephalin fractions was made (see p. 379).

Throughout all work with lipins it is advisable to keep all flasks, cylinders, etc., thoroughly flushed with nitrogen so that oxidation may, as far as possible, be prevented.

The N : P ratio of the lipin was nearly 1 : 1; the nitrogenous impurity which gives so much trouble in preparing lecithin from animal tissues does not seem to be present to any large extent.

Table II. *Analysis of lipin from yeast.*

	Theoretical figure for dioleyl-lecithin	Mixed lipin		$CdCl_2$-lecithin fraction	Kephalin fraction
% P	3·81	3·59	3·585	3·096	3·02
% N	1·72	1·87	1·44	1·79	1·38
N : P	1 : 1	1·15 : 1	0·89 : 1	1·28 : 1	1·01 : 1

Products of hydrolysis of the lipin.

The lipin is conveniently hydrolysed by boiling with 10 % sulphuric acid for 6 to 8 hours under a reflux condenser in a slow stream of nitrogen.

(a) *Nature of bases.* The aqueous hydrolysis liquid, after extraction with ether to remove fatty acids, was analysed to determine the total nitrogen (by Kjeldahl's method) and the amino-nitrogen (by the micro-method of Van Slyke). The proportion of choline to amino-ethyl alcohol can thus be estimated and the lecithin-kephalin ratio calculated.

The lipin from pressed yeast contained a larger proportion of kephalin than lecithin, whereas in that from "incubated" yeast they were present in nearly equal amounts. Only a small amount of material was available after purification and it was therefore impossible to effect a complete separation of lecithin from kephalin.

The following table shows the total and amino-nitrogen in the hydrolysis liquids together with the percentage composition of the lipin.

Table III.

	Lipin from pressed yeast		Lipin from incubated yeast			
	Without separation		Without separation		"Kephalin"	"Lecithin"
	(a)	(b)	(c)	(d)	(e)	(f)
Total N ...	0·1273 g.	0·0679 g.	0·0708 g.	0·1113 g.	0·0302 g.	0·0811 g.
Amino-N ...	0·1123 g.	0·0494 g.	0·0346 g.	0·0451 g.	0·0187 g.	0·0264 g.
Calculated						
% lecithin ...	27·3	11·8	51·15	59·5	37·96	67·5
% kephalin	72·7	88·2	48·85	40·5	62·04	32·5

Before its separation, the pressed yeast has been standing for some days in the wort and a considerable amount of autolysis has taken place. On the other hand, after the yeast has been incubated in the sugar-phosphate solution, from 80 to 90 % of the fatty matter has been freshly formed and this has only been standing in contact with the medium for a comparatively short time. In this freshly formed lipin it is interesting that the proportion of lecithin is greater than in the lipin derived from the pressed yeast. MacLean [1915] has shown that the proportion of lecithin present in the lipins extracted from the tissues is greater if the tissue is procured in as fresh a condition as possible and dried as quickly as possible. There seems some reason therefore to believe that both in yeast and in animal tissues autolytic changes may occur by which lecithin is converted into kephalin.

On the other hand yeast, when supplied with carbohydrate and phosphate but with no nitrogenous matter, has to use the substances stored in its own cells for its nitrogen supply and the relative amount of the two lipins formed may conceivably be influenced by this factor.

An experiment was carried out in which nitrogen was added to the medium in the form of ammonium sulphate. Unfortunately for our purpose the addition to the medium of a nitrogenous substance resulted in an increase of the total weight of yeast and in a diminution of the total weight of fat present. In this case, protein is made in preference to fat so that we can only investigate the case of increased fat formation when the nitrogen is obtained from substances originally present in the yeast cell.

(b) *Nature of acids.* The fatty acids from the hydrolysed lipin were extracted with purified ether immediately after cooling, and amounted to 50–60 % of the original material; the iodine value of an aliquot portion of the extract showed 60–70 % of the acids to be unsaturated.

The mixture was separated into the liquid unsaturated acid and the solid saturated acid fractions by the lead salt method as follows.

The acids were dissolved in a little alcohol, sufficient 2 % aqueous KOH was added to make the mixture neutral to phenolphthalein followed by 15 cc. of a hot 7 % lead acetate solution for every gram of fatty acid present. After cooling for some hours, the aqueous layer was poured off from the precipitated lead salts, which were washed with hot water and drained. These lead salts were then warmed with ether under a reflux until all the particles had been loosened from the sides of the flask, the whole cooled and filtered in an atmosphere of nitrogen, thereby giving ether-soluble and ether-insoluble fractions.

Treatment of ether-soluble lead salt fraction.

The ether-soluble lead salts were decomposed by shaking with dilute HCl and washing the ether solution with water till the washings were free from acid. After drying over sodium sulphate, aliquot portions were taken to determine the weight and iodine value of the acids present and for bromination.

The iodine value determined by Hübl's method was 90, corresponding with that of oleic acid.

The absence of acids of a greater degree of unsaturation, *i.e.* with more than one double bond, was confirmed by the bromination results.

The unsaturated fatty acids freed from moisture and ether were dissolved in sufficient pure carbon tetrachloride to give a 2 % solution and cooled to 0°. To this was added slowly at 0° a 2 % solution of bromine in carbon tetrachloride till the solution was just tinged red. After about 30 minutes an additional slight excess of the bromine solution was run in and the mixture allowed to stand overnight at 0°. The solvent and excess bromine were removed under reduced pressure at laboratory temperature and the residual oil treated with a small amount of dry light petroleum. In no case was there any trace of the precipitation of solid tetrabromide, and a portion of the bromination product without further treatment was found to contain 35·5 % bromine, corresponding to dibromostearic acid, which contains 35·9 % bromine.

It was found essential to exclude moisture and to allow the bromine to act only in small concentration, otherwise substitution products were formed which caused considerable trouble in the earlier stages of the work. The presence of insoluble hexa- and octo-bromides in the bromination products was never observed although small traces of a white insoluble material containing 14 % Br were found on two occasions; these were not identified.

Treatment of ether-insoluble lead salt fraction.

The insoluble material was treated either with dilute acetic or hydrochloric acid and shaken with ether to extract the liberated fatty acids and the ether solution was washed with water till the washings were neutral. After drying the ether solution over sodium sulphate, aliquot portions were taken for total weight, iodine value, molecular weight and melting-point determination.

It is most important that the I.V. of the "saturated" fatty acids be determined as it is almost impossible to obtain a quantitative separation of saturated fatty acids from unsaturated acids by the lead salt method when dealing with a mixture of a small proportion of saturated with a large proportion of unsaturated acid. A knowledge of the I.V. enables the amount of unsaturated acid still mixed with the saturated acid to be calculated, since oleic acid is the only unsaturated acid found in the lipin.

The molecular weight of the saturated acid was determined after recrystallising the acid from dilute alcohol, dissolving in 96 % alcohol and titrating against $N/10$ sodium hydroxide using phenolphthalein as indicator. Values were found lying between 251 and 260. The recrystallised acid melted at 55–57°.

Palmitic acid melts at 63° and has a molecular weight of 256. Since, however, a specimen of solid acid gave an I.V. of 20, some oleic acid was present and would account for the lowered melting-point and high molecular weight.

Examination of lecithin and kephalin fractions.

Further separation of the lipin into two fractions—the lecithin fraction and the kephalin fraction—was carried out by treating the lipin with excess of absolute alcohol. The residue insoluble in alcohol, which constituted the kephalin fraction, was filtered off and the solution treated with excess of 1 % alcoholic cadmium chloride solution to precipitate the insoluble lecithin-cadmium chloride complex. The compound formed by this addition was completely soluble in ether, yet it still contained one-third of its total nitrogen in the amino-form, indicating that the separation from kephalin was incomplete. The kephalin fraction previously separated by its insolubility in alcohol contained only two-thirds of its nitrogen as amino-nitrogen and was therefore a mixture of kephalin and lecithin in the ratio of 2 : 1. (See Table III.)

The only acids identified from the hydrolytic products of both fractions were palmitic and oleic acids and no evidence of the existence of any other acid was obtained.

The proportion of oleic acid was however greater in the acids derived from kephalin than in those derived from lecithin.

Table IV. *The products of hydrolysis of lipin.*

	Total lipin	Lecithin fraction	Kephalin fraction
Fatty acid %	61·0	64·0	62·0
I.V.	—	64·95	72·45
Fatty acids of ether-soluble lead salts			
(i) Yield %	(a) 53·0	(a) 70·0	(a) 81·0
	(b) 55·0	(b) 70·0	(b) 66·0
(ii) I.V.	(a) 89·97	(a) 75·9	(a) 68·3
	(b) 87·73	(b) 75·9	(b) 83·4
(iii) Br in bromination product %	(a) 39·5	(a) 35·51	(b) 35·42
	(b) 35·5	—	—
Theory for dibromostearic acid ...	35·9		
Fatty acids of ether-insoluble lead salts			
(i) Yield %	(b) 45·0	(a) 30·0	(b) 20
(ii) I.V.	(b) 41·0	54·96	61·9
(iii) Mol. wt.	(a) 251·4	260	259
	(b) 263·0	—	—
(iv) M.P.	(a) 53°	60°	58°
	(b) 54·5°	—	—
Calculated from I.V. since oleic is the only unsaturated acid present			
Unsaturated %	(b) 73·6	72·0	74·5
Saturated %	26·4	28·0	25·5
	100·0	100·0	100·0

As shown in Table IV, the fatty acids of yeast lipins consist of a little palmitic and much oleic acid.

The isolation of the saturated and unsaturated acids in the above experiments was unsatisfactory. The lead salt method of separation is known, however, to work badly when the mixture to be separated contains a large excess of the unsaturated constituent.

The only satisfactory method is by the fractional distillation of the methyl esters but for this a larger quantity of material must be available than we had at our disposal.

Combining the information derived from a knowledge of the I.V. and the amino-N : total N ratio, it is possible to calculate the composition of the yeast lecithin and kephalin, thus:

From nitrogen figures

kephalin fraction contains 62 % kephalin
38 % lecithin

lecithin fraction contains 67·5 % lecithin
32·5 % kephalin

I.V. of mixed fatty acids of

kephalin fraction: 72·45
lecithin fraction: 64·95

Let x be I.V. of mixed fatty acids of kephalin
,, y ,, ,, lecithin

Then $$62x + 38y = 7425$$
$$32{\cdot}5x + 67{\cdot}5y = 6495$$

Hence $$x = 82{\cdot}2$$
$$y = 56{\cdot}55$$

As only oleic and palmitic acids appear to be present
I.V. of kephalin acids is due to 91 % oleic acid
9 % palmitic acid
I.V. of lecithin acids is due to 62·6 % oleic acid
37·4 % palmitic acid

These results would suggest that yeast kephalin is a mixture of 82 % dioleyl-kephalin and 18 % oleyl-palmityl-kephalin, and the lecithin a mixture of 75 % oleyl-palmityl-lecithin and 25 % dioleyl-lecithin.

Examination of the "acetone-soluble" fat.

After separation of the lipin, the acetone solution was concentrated, reprecipitated with acetone and the filtered liquid again concentrated under reduced pressure at 40° till free from solvent. The residue consisted of the "acetone-soluble fat." It was a viscid, deep yellow oil which on standing in the cold room deposited white crystals of sterol or sterol ester.

Analysis of this fat showed the presence of only small amounts of phosphorus. The acid value remained fairly constant whereas the saponification value of fat from pressed and incubated yeast showed a distinct difference.

Table V. *Analysis of acetone-soluble fat from*

	(a) Pressed yeast				(b) Incubated yeast		
% P	0·091	0·062	0·048	0·345	0·252	0·075	0·114
A.V.		6·22			7·52	3·26	8·29
S.V.	172	175	176	172	122	128·8	137·2
I.V.		128·1				—	

Hydrolysis of fat.

After saponification of a sample of fat with alcoholic potash in a current of nitrogen, the alcohol was removed by heating on a water-bath and the residue dissolved in water. It was then extracted with ether to remove the unsaponifiable matter, acidified and again extracted to obtain the fatty acids.

(a) *Nature of acids.* As in the case of the lipin examination aliquot portions of the dry ether solution of the fatty acids were taken for iodine value, weight determination and the lead salts separation.

The lead salts soluble and insoluble in ether were separately decomposed as previously described and the liberated acids examined for iodine value, bromination products, molecular weight and melting point.

It was found that *the iodine value of the mixed acids was higher than that of the mixed lipin acids*, and on bromination, di- and tetra-bromo-derivatives were obtained which were separated by taking advantage of the sparing solubility of the tetrabromo-compound in light petroleum. It was found that the proportion of dibromide to tetrabromide was very variable.

Analysis showed that dibromostearic and tetrabromostearic acids were present, hence the existence of oleic and linolic acids in the original fat may be inferred. Palmitic acid was also isolated.

Table VI.

% fatty acid in fat		50·0–60·0
	(Mean of 5)	55·1
I.V. of mixed fatty acids		72·75–88·3
	(Mean of 6)	77·7
% total fatty acid present as unsaturated acid ...		50·0–56·0
	(Mean of 4)	51·5
I.V. of mixed unsaturated acids		97·1–119·6
	(Mean of 4)	107·8
% bromine in dibromo-acid		34·95
		35·50
		36·05
Theory for dibromostearic acid		35·9
% bromine in tetrabromo-acid		53·46
Theory for tetrabromostearic acid		53·33
% total fatty acid present as saturated fatty acid		48·0–50·0
	(Mean of 2)	49·0
I.V. of saturated acids		24·0
M.P. of recrystallised acid		55°
		57·5°
		63°
M.P. of palmitic acid		63°
Molecular weight		249–258
	(Mean of 3)	253·7
Theory for palmitic acid		256

(b) *The unsaponifiable fraction.* (See Table VII.) The unsaponifiable material was examined for the presence of ergosterol by warming with light petroleum and recrystallising the soluble material from alcohol. A beautiful white crystalline solid was obtained with an iodine value about 200; M.P. 140–145°. The substance crystallised in characteristic elongated hexagonal plates.

The free sterol in the fat was determined by dissolving 0·4 g. in 50 cc. of 95 % alcohol and adding 50 cc. of a hot 1 % solution of digitonin in 90 % alcohol. After standing a few hours the digitonin-sterol compound was filtered through a Gooch crucible and dried at 110° before weighing. The total sterol was estimated by first hydrolysing 0·4 g. of fat, extracting the unsaponifiable material and precipitating with digitonin as above.

It was found that the fat contained 1 % free ergosterol and some 14 % combined sterol, and that this sterol constitutes 50 % of the total unsaponifiable material.

Since the iodine value of the total unsaponifiable matter is approximately half that of the ergosterol, it follows that the remainder of the unsaponifiable matter must consist almost entirely of saturated compounds, which we are now further investigating.

Table VII.

	Smedley-MacLean and Thomas [1920]	Smedley-MacLean and Daubney	
% unsaponifiable matter in fat	39·8	24·3–33·35	
		Mean of 6	29·6
I.V.	—	95·81	
% ergosterol in fat	17·23	15·63	
I.V.	177·1	188·5	200·1
M.P.	—	140–5°	140–5°
% free ergosterol	4·9	1·24	
% combined ergosterol ...	12·3	14·19	
% ergosterol in unsapon. material	43·3	52·7	

Summary and discussion of results.

(1) It has previously been shown that the effect of adding alkali phosphates to a well oxygenated solution of glucose in which yeast is incubated is approximately to double the total amount of ether-soluble (lipoid) substance. At the same time the proportion of lipin is somewhat diminished but the total quantity is much increased. The small amount of nitrogen necessary to form the new lipin must be derived from the nitrogenous material previously existing in the yeast. The phosphorus is presumably derived from the phosphate: the total amount of phosphorus in the yeast is very largely increased and evidence has already been furnished that the phosphate is taken up in association with carbohydrate. No significant variation in the proportion of unsaponifiable matter was observed.

It appears therefore that the addition of phosphate to a sugar solution increases the formation of fat, lipin and sterol and that, as in the animal organism, these lipoid constituents maintain a fairly constant balance.

(2) The phospholipins of yeast consist of lecithin and kephalin and in both of these the same fatty acid radicals, oleyl and palmityl, appear to be present. The proportion of oleyl is apparently greater in the kephalin than in the lecithin molecule. There seems to be some evidence that the proportion of kephalin is higher in lipin from yeast that has undergone partial autolysis than in the freshly produced lipin—a result in accordance with the findings of MacLean for animal tissues. The saponification value (175) of the lipoid matter from the pressed yeast obtained after the yeast had stood for some time in contact with the wort was considerably higher than that (130) of fatty matter from the yeast which had been incubated in the glucose-phosphate medium.

(3) Perhaps the most interesting result established by the experiments now described is that the fatty acids which occur as constituents of the phospholipins of yeast have a lower iodine value than the fatty acids of the acetone-soluble fat. Few reliable data exist for comparing the degree of unsaturation of the acids from the lipins and the acetone-soluble fat respectively of the same organ. The acids of the liver were examined by Kennaway and

Leathes [1909] who found that though the highly unsaturated acids were not confined in the liver to the lipins, yet generally speaking the acids of the liver were more unsaturated than those of the simple glycerides. A similar relation was shown by Bloor [1926] to hold for the fatty acids of the heart. The iodine value of the acids from the heart lipins is very considerably higher than that of the acids from the non-phosphorised fat. Arachidonic acid, a twenty-carbon acid with four double bonds, constitutes 6 % of the lipin acids but only 2 % of the acids from the simple glycerides.

On the other hand, when Bloor [1924] examined the fatty acids which occur in the blood he found that, contrary to expectation, the more highly unsaturated acids for the most part did not occur in the lipins of the blood but were associated with the sterols.

Yeast fat is characterised by the very large proportion of unsaponifiable matter which it contains. This may constitute one-third by weight of the whole fat: approximately half of the unsaponifiable matter consists of the sterol characteristic of the fungi, ergosterol, and nearly the whole of this is in combination with fatty acids as esters. The remainder of the unsaponifiable matter consists of a saturated yellow oil which is being further investigated. In yeast it is the portion of fat containing steryl and glyceryl esters from which the linolic acid is derived. In the yeast lipins oleic acid seems to be the only unsaturated acid present.

(4) Another point of interest about the yeast lipins is that they occur in a less complicated mixture than is usually found in other tissues. In both the kephalin and lecithin fractions from yeast, oleic and palmitic acids appear to be the only two acids present and of these oleic acid is present in very much larger amount. We may conclude therefore that dioleyl- and palmityl-oleyl lecithins and kephalins occur in yeast and that the dioleyl compounds are present in the greater amount. The proportion of the oleic acid is somewhat greater in the kephalin than in the lecithin fraction. In the acetone-soluble fat, on the other hand, saturated and unsaturated acids seem to be present in approximately equal proportions. Very little information is available as to the nature of the acids present in plant lipins. The lipins of the Soya bean were investigated by Levene and Rolf [1925, 1, 2; 1926] who showed that the iodine value of the mixed lipin acids was low compared with that of the mixed lipin acids from animal tissues: the percentage of saturated acid was also exceptionally low. The unsaturated acids isolated from the lipins all contained eighteen carbon atoms and comprised oleic, linolic and linolenic acids. The saturated acids accounted for 15 %, the unsaturated for 43 % and the acids constituting the remaining 42 % were not satisfactorily identified, though some evidence was obtained of the existence of an unsaturated hydroxy-acid. In the work of other observers on various vegetable lipins, oleic acid appears to have been the only acid identified. There are no data available from which a comparison of the acids from the lipins and acetone-soluble fat of the same plant can be made.

On the whole, from such evidence as is available, it seems that the acids of the vegetable lipins have a lower degree of unsaturation than the acids derived from the animal lipins and they are also characterised by a low proportion of saturated acid. In some cases at any rate unidentified acids of unknown structure appear to be present.

Arachidonic acid has not so far been shown to occur in the lipins of the plant kingdom, linolenic acid being the most unsaturated acid yet isolated. Further data as to the nature of the acids present in plant lipins are much needed; a comparison of the acids which occur in the animal and plant lipins may possibly help us to understand something of the functions of the lipins themselves.

Certainly in the yeast lipins, the radicals of highly unsaturated acids seem to be absent and the doubly unsaturated acid of yeast fat occurs only in combination with sterol or glycerol.

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